Food Control 26 (2012) 269–273

Contents lists available at SciVerse ScienceDirect

Food Control

journal homepage: www.elsevier.com/locate/foodcont

Mixed culture enrichment of *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella enterica*, and *Yersinia enterocolitica*

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ARTICLE INFO

Article history:
Received 18 September 2011
Received in revised form
17 January 2012
Accepted 21 January 2012

Keywords:
Co-enrichment
E. coli O157:H7
Listeria monocytogenes
Mixed culture
Salmonella enterica
Yersinia enterocolitica

ABSTRACT

Rapid methods for testing foods for the presence of pathogenic bacteria typically suffer from poor sensitivity and therefore require large concentrations of the bacteria to be present for detection. Food contaminated with pathogenic bacteria may often contain only a very small number of the microorganisms making their direct detection very challenging even with existing state-of-the-art methods. Therefore prior to detection, it may be of pertinence to increase the number of potentially present pathogenic bacteria through growth in an appropriate culture medium. Furthermore, multiplexed testing for the presence of different bacteria in food samples necessitates the ability to simultaneously increase, through growth/culture, the concentration of each targeted bacterial pathogen to a detectable level. We have evaluated several commercially available and custom media preparations for their ability to support the simultaneous growth of the following bacteria: *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella enterica*, and *Yersinia enterocolitica*. Growth conditions (primarily enrichment media formulation and incubation temperature) that resulted in multiplication of all four pathogens to ca. 1×10^5 cells/mL within 24 h or less were considered sufficient as a culture enrichment step prior to testing with most rapid methods. Axenic culture enrichment of all the bacteria for 18 h readily yielded concentrations significantly greater than 1×10^5 cells/mL for each of 5 different growth media. Mixed culture enrichment of the bacteria in pristine culture media and ground pork slurries indicated that several of the tested conditions appeared to be suitable for the growth of the selected bacteria to the targeted detection level, with the exception of *L. monocytogenes* in the ground meat (inoculated at 1.1 CFU/mL).

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1. Introduction

Methods developed for the rapid, multiplexed detection of various species of pathogenic bacteria have been developed over the years and include multiplex polymerase chain reaction (Fratamico & Strobaugh, 1998), immunomagnetic electrochemistry with rotating disc electrochemical detection (Rishpon, Gezundhajt, Soussan, Rosen-Margalit, & Hadas, 1992), time-resolved fluorimetry (Tu, Golden, Andreotti, & Irwin, 2002), electrochemiluminescence (Yu & Bruno, 1996), and antibody-based microarray (Delehanty & Ligler, 2002). Though rapid methods yield results on a time-scale of minutes to hours as opposed to days required by traditional culture methods, these methods typically do not exhibit limits of detection better than the 1×10^2 to 1×10^3 cells (or CFU) of targeted bacterial analyte per milliliter. Such "real-time" limits of detection, in cells/mL, have been demonstrated to be ca. 1×10^3 cells/mL for antibody-direct epifluorescent filtration technique (Tortorello & Gendel, 1993), 5×10^2 CFU/mL for enzyme-linked immunofiltration assay (Paffard, Miles, Clark, & Price, 1997), 7.6×10^3 cells/mL enzyme-linked immunomagnetic chemiluminescence (Gehring et al., 2004), 4.7×10^3 cells/mL enzyme-linked immunomagnetic electrochemistry (Gehring, Brewster, Irwin, Tu, & Van Houten, 1999), ca. 5×10^3 cells/mL for filtration–immunoelectrochemistry (Brewster & Mazenko, 1998), ca. 7.1×10^2 cells/mL for immunoligand assay–light addressable potentiometric sensing (Gehring, Patterson, & Tu, 1998), 1×10^3 cells/mL for immunomagnetic electrochemiluminescence (Yu & Bruno, 1996), and 1×10^2 cells/mL for immunomagnetic separation–fluorescence microscopy (Tu, Uknalis, Patterson, & Gehring, 1998). Though remarkable, all

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0956-7135/$ – see front matter Published by Elsevier Ltd.
doi:10.1016/j.foodcont.2012.01.047

of these reported detection limits are well above the typical level of pathogen contamination in foods. Therefore, rapid methods for pathogen detection are generally preceded by culture enrichment prior to analysis.

In the development of rapid methods that are capable of multiplexing, it is of merit to compare available media intended for the enrichment of mixed cultures. In this study, the detection limit of 1×10^5 CFU/mL was conservatively selected as a target cell enrichment concentration for analysis and/or development of mixed culture growth methods. For mixed culture growth, media formulations, such as the Universal Pre-enrichment Broth (UPB; (Bailey & Cox, 1992; Bhaduri & Cottrell, 2001)) and SEL broth (Kim & Bhunia, 2008) were compared with traditionally non-selective broths including buffered peptone water (BPW) and trypticase soy broth (TSB) as well as Buffered Listeria Enrichment Broth (BLEB).

Pure and mixed cultures of *E. coli* O157:H7, *L. monocytogenes*, *Salmonella enterica*, and *Yersinia enterocolitica* were incubated overnight in select, pristine growth media as well as the food matrix, ground pork, and numerically assessed for total number of live cells as reported in colony forming units (CFU) per unit volume. Individual cell growth concentrations were reported for the various culture conditions that were assessed.

2. Material and methods

2.1. Materials

Bacterial strains used in this research included: *E. coli* O157:H7, strain B1409 and *S. enterica* serovar Typhimurium, strain G8430 (Centers for Disease Control, Atlanta, GA), *L. monocytogenes* 1/2a, strain 19111 (American Type Culture Collection, Manassas, VA), and *Y. enterocolitica* O:8, strain WA-08 (Food and Drug Administration, Rockville, MD). Culture media included Brilliant Green Bile Broth 2% (BGB), Buffered Listeria Enrichment Broth Base (BLEB), Buffered Peptone Water (BPW), Trypticase Soy Broth (TSB), and Universal Pre-enrichment Broth (UPB) (Becton, Dickinson and Company, Sparks, MD). Plating media included Plate Count Agar (PCA), CHROMagar O157, CHROMagar Listeria, CHROMagar Salmonella, and CIN agar (Becton, Dickinson and Company). Other materials included phosphate buffered saline tablets (PBS) (Sigma-Aldrich, St. Louis, MO), and Stomacher bags (Fisher Scientific; Pittsburgh, PA). Note, BLEB base was used without selective agents, as to not inhibit the growth of the other bacteria in the mixed culture. Other chemicals used were of reagent grade. Pork loin, obtained from a local supermarket, was trimmed to remove surface contamination and the interior meat was ground in a meat grinder under aseptic conditions. The ground pork was placed into sterile stomacher bags that were stored at −20 °C until use.

2.2. Apparatus

Enumeration of bacterial cells was conducted using a Petroff-Hausser counting chamber (Hausser Scientific, Horsham, PA). Harvesting of cells was done using an Eppendorf 5417R centrifuge (Eppendorf, Westbury, NY). All reactions with vortexing were performed on a Vortex-Genie (Scientific Industries, Bohemia, NY). Sample mixing, incubation, shaking, and enrichment of cultures, samples, and plates were performed in the following: Stomacher (Seward Medical Ltd, London, United Kingdom), Innova 4000 shaking incubator (New Brunswick Scientific, Edison, NJ), Labnet 311DS shaking incubator (Labnet International, Edison, NJ), and Fisher Isotemp 300 series static incubator (Fisher Scientific, Pittsburgh, PA), respectively.

2.3. Bacterial growth and enumeration

Bacterial strains were stored frozen at −70 °C in 20% glycerol. One loopful (~10 μL) from each frozen stock was cultured for 18 h at 37 °C in 25 mL of broth (type of broth varied by experiment) with shaking at 160 rpm. *L. monocytogenes* was also incubated at 30 °C in each type of broth.

The efficacy of each organism to grow independently in the test broths was determined. Aliquots (0.1 mL) of serially diluted overnight cultures were spread plated onto CHROMagar O157, CHROMagar Listeria, CHROMagar Salmonella, and CIN agar. Plates were incubated at 37 °C for 24 h, after which colonies were enumerated, and the CFU/mL was calculated for each bacterial species. Each strain and test broth combination was independently replicated at least three times.

Using the same overnight cultures used above for gauging pure culture growth efficacy, the total cell concentration of each bacterial strain was estimated using a Petroff-Hausser counting chamber to enable a uniform inoculation of 50 cells/mL for subsequent mixed culture enrichment. Briefly, portions (1 mL) of the cells were harvested by centrifugation at 5000 rpm for 5 min and the pellet was re-suspended in 1 mL PBS. The re-suspended cultures were then diluted 1:100 in PBS. An aliquot (6 μL) was added to the counting chamber and the center 0.2 mm × 0.2 mm grid was counted. The counting chamber was thoroughly washed with 70% ethanol, and the counting procedure was repeated three more times for a total of four replicates. The average of the four replicates was used to determine the cell concentration for each culture.

In addition to using the Petroff-Hausser counting method to establish the total cells/mL for each culture, aliquots were also plated onto PCA to determine the CFU/mL. After overnight (16–18 h) incubation at 37 °C, bacterial colonies were enumerated and the CFU/mL determined. The concentration of the live cultures was reported as an average, ±standard deviation, of these amounts. One-way analysis of variance (ANOVA) of the replicated enumerations was employed to compare growth of *L. monocytogenes* at 30 and 37 °C. The ratio of CFU/mL to total cells/mL was to determine the percentage of live cells. This information was subsequently used when inoculating ground pork at very low levels.

2.4. Mixed culture enrichment

Each bacterial culture was diluted in PBS and inoculated into 100 mL of broth contained in stomacher bags to obtain a final concentration of 50 cells/mL. The sample was manually mixed for ~10 s to ensure an even distribution. The flaps of the stomacher bags were secured but not sealed with tape (thus leaving the bag open for gas exchange) and placed, unless otherwise indicated, into a shaking incubator at 30 °C for 18 h at 160 rpm.

2.5. Inoculation of ground pork

Pork tenderloin was purchased from a local supermarket, aseptically ground in a biological safety cabinet, divided into 25 g portions contained in stomacher bags, and frozen for subsequent experiments. Prior to experimentation, pork samples were thawed at 4 °C overnight. Each bacterial strain was diluted in PBS and a 1 mL aliquot was co-inoculated into the pork samples, to yield final concentrations of 0.2 cell/mL of *E. coli* and *Salmonella*, 1 cell/mL of *Y. enterocolitica*, and 2 cells/mL of *L. monocytogenes* in a 250 mL sample. A separate 25 g portion of ground pork, inoculated with 4 mL PBS, served as a control. The inoculum was manually "massaged" into the pork for ~10 s to ensure an even mixture. To the inoculated pork, 225 mL of broth was added and mixed in a Stomacher for 30 s. An aliquot of the control sample was

Table 1
Constituents of employed enrichment broths.

Medium	Nutrient(s)	Buffer(s)	Salt(s)	Supplement(s)	pH[a]
BGB	Peptone, lactose	–	–	Oxgall, brilliant green	7.2
BLEB	Casein digest, soybean digest, dextrose, yeast extract, sodium pyruvate	Phosphate	NaCl	(Recommended agents not added)	6.9
BPW	Peptone	Phosphate	NaCl	(None)	7.0
SEL	Pancreatic digest of casein, yeast extract, dextrose, soytone, sodium pyruvate	Phosphate	NaCl	Acriflavine, cycloheximide, fosfomycin, nalidixic acid	7.0
TSB	Peptone from casein, peptone from soymeal, D(+)glucose	Phosphate	NaCl	(None)	7.3
UPB	Casein digest, soybean digest, dextrose, protease peptone, sodium pyruvate	Phosphate	NaCl, Mg_2SO_4, ferric ammonium citrate	(Recommended antibiotics not added)	6.1

[a] At RT.

plated onto the selective agars as listed above and PCA to determine if any target organism was present in the pork, and to establish the level of background organisms pre-enrichment. The flaps of the stomacher bags were secured but not sealed with tape (thus leaving the bag open for gas exchange) and placed into a shaking incubator at 30 °C for 24 h at 160 rpm.

2.6. Post-assay enumeration

After enrichment, serial dilutions (in PBS) of the enriched mixed culture samples were spread plated on to the selective agars listed above to yield a target concentration of 100–300 colonies per plate. The level of background flora and potential target organisms in the control sample was again determined post-enrichment following the same procedure as pre-enrichment. The plates were incubated overnight at 37 °C, and resultant colonies were enumerated. The concentration of the live cultures was reported as an average, ±standard deviation, of these amounts.

3. Results and discussion

3.1. Axenic growth of bacteria in various enrichment broths

Prior to multiplex enrichment, the efficacy of each enrichment broth to support the growth of the target organisms without competition was established. The constituents and pH of the tested enrichment broths are shown in Table 1. The results presented in Fig. 1 exhibit that all bacteria tested grew to a concentration that was significantly greater than 1×10^5 CFU/mL, a concentration detectable by the vast majority of rapid methods. Interestingly, BGB, which is formulated to confirm the presence of coliforms and suppress the growth of other Gram positive as well as many Gram negative bacteria other than coliforms, readily supported the growth of all bacteria tested including *L. monocytogenes*, a Gram positive bacteria, although at a lower concentration than the others.

In addition to enrichment broth, *L. monocytogenes* was also tested at 37 °C and 30 °C, two common incubation temperatures used for enrichment (also shown in Fig. 1). A one-way ANOVA was used to determine if the growth of *L. monocytogenes* at 30 °C was significantly different than at 37 °C. The results showed that in BLEB and BPW the growth was significantly better ($P < 0.05$) for *L. monocytogenes* at 30 °C as opposed to 37 °C. No significant differences were found among the other broths.

3.2. Growth of bacteria in mixed culture in pristine growth media

Unlike the experiments performed with culturing the target bacteria in pure culture, this experiment, with mixed cultures, fully controlled quantitation of bacterial numbers at multiple stages of testing, starting with an initial inoculum concentration of 50 cells/mL. In addition, SEL broth was added due to its promising results for enriching mixed cultures (Kim & Bhunia, 2008) and BGB was removed from further testing since the other broths yielded better growth for *L. monocytogenes*.

Enrichment broths were co-inoculated with *E. coli*, *S. enterica*, *L. monocytogenes*, and *Y. enterocolitica* each at 50 cells/mL and incubated at both 37 °C and 30 °C. Again, there was an better growth at 30 °C compared with 37 °C. Each organism grew at least 45% more at 30 °C than at 37 °C in all test broths expect for *E. coli* in BLEB and BPW (data not shown). A comparison of the growth of

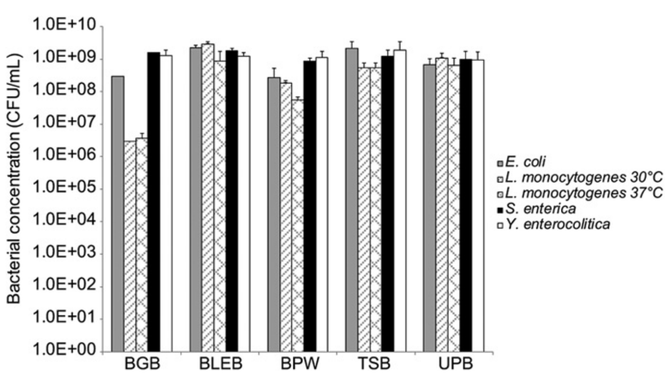

Fig. 1. Axenic growth of bacteria in various enrichment broths. Bacterial strains were individually enriched in each test broth at 37 °C for 18 h. *L. monocytogenes* was tested at both 30 °C and 37 °C. Key: gray = *E. coli* O157:H7, striped = *L. monocytogenes* ½a (30 °C), squares = *L. monocytogenes* ½a (37 °C), black = *S. enterica*, and white = *Y. enterocolitica* O:8. Error bars denote the standard deviation among three replicates.

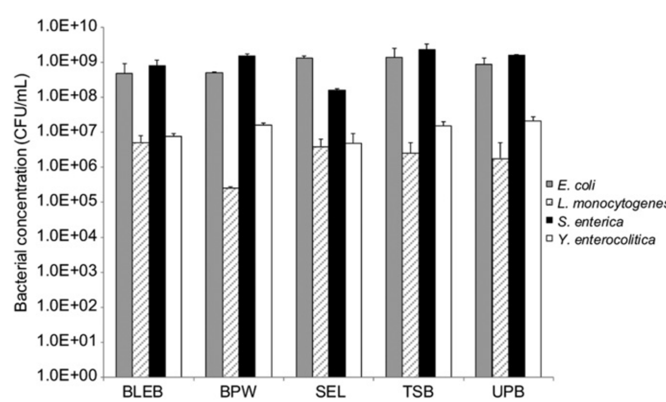

Fig. 2. Comparison of pristine enrichment media with mixed cultures. Bacterial cultures were serially diluted to ca. 50 cells/mL in PBS and co-enriched for 18 h at 30 °C. Key: gray = *E. coli* O157:H7, striped = *L. monocytogenes* ½a (30 °C), black = *S. enterica*, and white = *Y. enterocolitica* O:8. Error bars denote the standard deviation among three replicates.

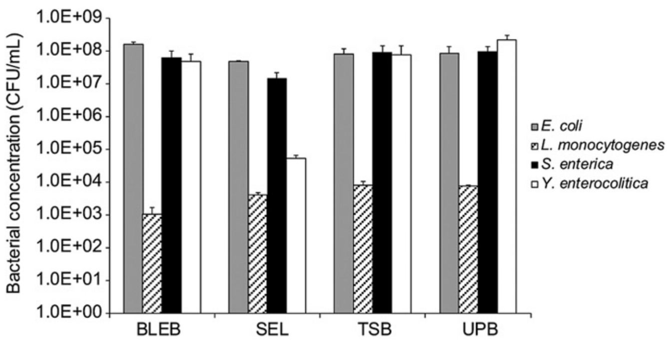

Fig. 3. Comparison of mixed cultures in enrichment media containing food matrix (ground pork). Serially diluted portions of overnight cultures were mixed and co-enriched for 24 h at 30 °C in the various enrichment media containing ground pork. Concentrations of individual bacterial populations present in the mixed cultures were reported above as CFU/mL. Key: gray = *E. coli* O157:H7, striped = *L. monocytogenes*, black = *S. enterica*, and white = *Y. enterocolitica*; Initial inocula were 0.16 ± 0.012, 1.1 ± 0.000, 0.18 ± 0.006, and 0.41 ± 0.035 CFU/mL for the respective bacteria.

each organism in mixed culture at 30 °C is shown in Fig. 2. An analysis of variance revealed significant differences ($P < 0.05$) in the growth of *S. enterica*, *L. monocytogenes*, and *Y. enterocolitica* among the test broths. The results of the plate counts indicate that there was considerable evidence of stunted growth for some of the bacteria, typically *L. monocytogenes* and *Y. enterocolitica* in mixed culture. It would appear that *L. monocytogenes* and *Y. enterocolitica* are more susceptible to competition and therefore their growth was inhibited in the presence of *E. coli* O157:H7 and *S. enterica* during co-enrichment. Regardless, all of the 18 h growth conditions yielded enrichment of each bacterium to a concentration greater than 1×10^5 CFU/mL.

3.3. Growth of bacteria in mixed culture in ground pork

In order to demonstrate practical application, these broths were used to enrich mixed cultures inoculated into a test food sample. Analogous to the mixed culture study in pristine growth media, growth of mixed cultures in media containing incurred ground pork was also assessed. Lower concentrations of pathogenic bacteria were inoculated into the pork to simulate low levels of contamination potential in real world samples. The inoculated ground pork was enriched at 30 °C, since the mixed culture results showed an increase in growth at 30 °C as compared with 37 °C.

Similar to the results with the mixed cultures in pristine growth media, stunted growth again was observed for some of the bacteria in the presence of ground pork but to an even greater extent (data not shown). Therefore, the incubation time was increased from 18 to 24 h to allow more detectable levels to be achieved. For this experiment, four 24 h, 30 °C growth conditions including BLEB, SEL, TSB, or UPB growth media, all yielded enrichment of all bacteria to a concentration of ca. 1×10^5 CFU/mL or greater, with the exception of *L. monocytogenes* in all broths and *Y. enterocolitica* in SEL (Fig. 3). Results from the control samples showed the levels of background flora ranged from an initial 5.8×10^2 to 4.5×10^3 CFU/mL to a post-enrichment range of 1.3×10^8 to 1.3×10^9 CFU/mL. No target organisms were present in the control samples.

4. Conclusion

Several growth media tested were demonstrated to co-enrich all of the tested pathogens (*E. coli* O157:H7, *L. monocytogenes*, *S. enterica*, and *Y. enterocolitica*) to ca. 1×10^5 CFU/mL in an overnight (18 h) mixed culture at 30 °C in pristine media. However, there is a disparity between reporting bacterial concentrations in cells or CFU per mL, especially given that immunoassay-based rapid methods do not discern live versus dead bacteria, the target enrichment concentration of 1×10^5 CFU/mL was considered acceptable as a substitute for 1×10^5 cells/mL. This rationalization particularly holds true when it is understood that any subpopulation of dead cells, which inevitably occurs in bacterial cultures, would preclude that the number of CFU would always be less than number of cells. Hence, reporting an acceptable growth target concentration in CFU/mL is more conservative than cells/mL.

It was observed in mixed culture enrichment a marked difference in growth among the bacteria tested. *L. monocytogenes* and *Y. enterocolitica*, had overall growth densities considerably less in mixed culture than in axenic culture. This phenomenon has been documented in other studies. The ability of a dominant species to halt the growth of other organisms when it reaches stationary phase was documented with *Salmonella* spp. by Jameson (1962). The likely consensus is that the dominant species is responsible for considerable depletion of available nutrients and change in the pH of the enrichment broth. Usually, the dominant species is based on highest inoculum, or high density of background (Gnanou Besse et al., 2006). However Mellefont, McMeekin, and Ross (2008) observed that even when *L. monocytogenes* was inoculated at higher concentrations than *E. coli*, the *E. coli* was not suppressed by the *L. monocytogenes* even though it was the dominant species. Our observations in ground pork support this, as the inoculation level of *L. monocytogenes* was higher than that off all the other test organisms, albeit at very low densities.

Concurrent growth (mixed cultures) of all four bacteria in several of the pristine culture media was observed to occur at levels that were consistently higher than those observed for mixed culture in the food matrix, ground pork, indicating that there may have been either growth inhibition by the food (released bacteriostatic agents or more likely competition with meat-associated background flora) or binding to food particles. Al-Zeyara, Jarvis, and Mackey (2011) observed the inhibition of *L. monocytogenes* by natural background flora in several different food matrices. It was also shown in the same study that some of this suppression was overcome with enrichment in selective broths.

Use of these media and growth conditions should increase the concentration of targeted bacteria sufficiently to facilitate subsequent detection with rapid, multiplexed detection methods. Furthermore, concurrent growth, of potentially contaminant pathogens in food samples, in a single reaction vessel (e.g. stomacher bag or flask) would streamline the food safety screening processes thus reducing labor and expense of disposables. It would appear that, if ground pork samples are enriched, only those rapid methods with the lowest limits of detection must be employed for testing. Since only a small volume of the enriched samples typically needs to be used for rapid screening, culture enriched samples that are determined to be positive for pathogens may be further subjected to acceptable identification and/or confirmation methods (e.g., selective plate culture and/or biochemical testing, 16S rDNA sequencing, pulsed field gel electrophoresis, ribotyping, multiplex PCR, etc.).

Acknowledgments

We gratefully thank Drs. Chin-Yi Chen, Peter Irwin, and George Paoli, from the Eastern Regional Research Center, Agricultural Research Service, U. S. Department of Agriculture, for helpful discussions. We thank Barbara Drysdale (United Healthcare, Inc.) for her critical reading of this manuscript. Mention of brand or firm names does not constitute an endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

References

Al-Zeyara, S. A., Jarvis, B., & Mackey, B. M. (2011). The inhibitory effect of natural microflora of food on growth of Listeria monocytogenes in enrichment broths. *International Journal of Food Microbiology, 145*(1), 98–105.

Bailey, J. S., & Cox, N. A. (1992). Universal preenrichment broth for the simultaneous detection of Salmonella and Listeria in foods. *Journal of Food Protection, 55*, 256–259.

Bhaduri, S., & Cottrell, B. (2001). Sample preparation methods for PCR detection of Escherichia coli O157:H7, Salmonella typhimurium, and Listeria monocytogenes on beef chuck shoulder using a single enrichment medium. *Molecular and Cellular Probes, 15*(5), 267–274.

Brewster, J. D., & Mazenko, R. S. (1998). Filtration capture and immunoelectrochemical detection for rapid assay of *Escherichia coli* O157:H7. *Journal of Immunological Methods, 211*(1–2), 1–8.

Delehanty, J. B., & Ligler, F. S. (2002). A microarray immunoassay for simultaneous detection of proteins and bacteria. *Analytical Chemistry, 74*(21), 5681–5687.

Fratamico, P. M., & Strobaugh, T. P. (1998). Simultaneous detection of Salmonella spp and Escherichia coli 0157:H7 by multiplex PCR. *Journal of Industrial Microbiology and Biotechnology, 21*(3), 92–98.

Gehring, A. G., Brewster, J. D., Irwin, P. L., Tu, S., & Van Houten, L. J. (1999). 1-Naphthyl phosphate as an enzymatic substrate for enzyme-linked immunomagnetic electrochemistry. *Journal of Electroanalytical Chemistry, 469*(1), 27–33.

Gehring, A. G., Irwin, P. L., Reed, S. A., Tu, S.-I., Andreotti, P. E., Akhavan-Tafti, H., et al. (2004). Enzyme-linked immunomagnetic chemiluminescent detection of *Escherichia coli* O157:H7. *Journal of Immunological Methods, 293*(1–2), 97–106.

Gehring, A. G., Patterson, D. L., & Tu, S. I. (1998). Use of a light-addressable potentiometric sensor for the detection of *Escherichia coli* O157:H7. *Analytical Biochemistry, 258*(2), 293–298.

Gnanou Besse, N., Audinet, N., Barre, L., Cauquil, A., Cornu, M., & Colin, P. (2006). Effect of the inoculum size on *Listeria monocytogenes* growth in structured media. *International Journal of Food Microbiology, 110*(1), 43–51.

Jameson, J. E. (1962). A discussion of the dynamics of Salmonella enrichment. *Epidemiology and Infection, 60*(02), 193–207.

Kim, H., & Bhunia, A. K. (2008). SEL, a selective enrichment broth for simultaneous growth of *Salmonella enterica*, *Escherichia coli* O157:H7, and *Listeria monocytogenes*. *Applied and Environmental Microbiology, 74*(15), 4853–4866.

Mellefont, L. A., McMeekin, T. A., & Ross, T. (2008). Effect of relative inoculum concentration on Listeria monocytogenes growth in co-culture. *International Journal of Food Microbiology, 121*(2), 157–168.

Paffard, S. M., Miles, R. J., Clark, C. R., & Price, R. G. (1997). Amplified enzyme-linked-immunofilter assays enable detection of 50-105 bacterial cells within 1 hour. *Analytical Biochemistry, 248*(2), 265–268.

Rishpon, J., Gezundhajt, Y., Soussan, L., Rosen-Margalit, I., & Hadas, E. (1992). In P. R. Mathewson, & J. W. Finley (Eds.), *Chapter 6-Immunoelectrodes for the detection of bacteria. ACS symposium series No. 511*, (pp. 59–72).

Tortorello, M. L., & Gendel, S. M. (1993). Fluorescent antibodies applied to direct epifluorescent filter technique for microscopic enumeration of *Escherichia coli* O157:H7 in milk and juice. *Journal of Food Protection, 56*(8), 672–677.

Tu, S., Golden, M., Andreotti, P., & Irwin, P. (2002). The use of time-resolved fluoroimmunoassay to simultaneously detect *Escherichia coli* O157:H7, *Salmonella enterica* Serovar Typhimurium and *Salmonella enterica* Serovar Enteriditis in foods. *Journal of Rapid Methods and Automation in Microbiology, 10*(1), 37–48.

Tu, S., Uknalis, J., Patterson, D., & Gehring, A. (1998). Detection of immunomagnetically captured, 4′6′-diamidino-2-phenylindole (DAPI)-labeled *E. coli* O157:H7 by fluorescent microscope imaging. *Journal of Rapid Methods and Automation in Microbiology, 6*(4), 259–276.

Yu, H., & Bruno, J. (1996). Immunomagnetic-electrochemiluminescent detection of *Escherichia coli* O157 and *Salmonella typhimurium* in foods and environmental water samples. *Applied and Environmental Microbiology, 62*(2), 587–592.